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14. ABSTRACT  The major goal of this project is to identify small inhibitory peptides that can target critical DNA damage responsive pathways in order to develop novel therapeutic agents for prostate cancer radiotherapy. Previously we have demonstrated that small fusion peptides containing SMC1 phosphorylation and NBS1 ATM binding sequences can inhibit ATM activity and decrease prostate tumor cell clonogenic survival after radiation, therefore these peptides can be tested for in vivo activities of radiosensitization. In order to achieve this goal, we generated a series of tumor homing peptides containing these sequences and proved tumor specific targeting of the peptides. Ongoing experiments include approaches to reduce radiation damage to normal tissues and to evaluate of the fusion peptides as radiosensitizers in prostate cancer xenograft models.					
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## INTRODUCTION

The goal of this project is to identify and characterize small inhibitory peptides that can sensitize prostate tumor cells to radiation therapy both *in vitro* and *in vivo*. We aim to target critical DNA damage responsive pathways. Since that last two performance period, we have identified several peptides that can interfere with ATM-mediated pathways. ATM is a protein kinase critical for the cellular survival after radiotherapy. ATM phosphorylates a series targets including the Structural Maintenance of Chromosome protein one (SMC1)(Kim et al., 2002; Kitagawa et al., 2004; Yazdi et al., 2002) and Nijmegen Breakage Syndrome 1 (NBS1) (Lim et al., 2000) to limit the amount of radiation sensitivity. We have developed several peptides that function as radiosensitizers *in vitro* by targeting ATM phosphorylation of SMC1 and NBS1-ATM interaction (Cariveau et al, 2007). Therefore Task 1 and 2 of this project have been completed. In this performance period, we initiated *in vivo* studies (Task 3) to test the efficacy of the peptides as powerful radiosensitizers in a prostate xenograft model.

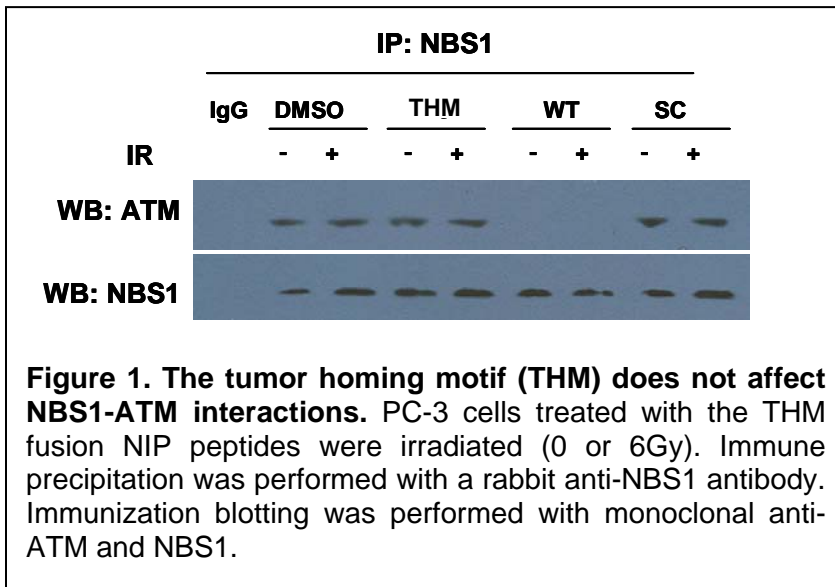
## BODY

### Task 3. To study radiosensitizing effects of the peptides on a prostate cancer xenograft model in nude mice.

#### 1. Development of the THM-peptides for *in vivo* studies.

We first generated a set of fusion peptides containing the CNGRC tumor homing motif (THM) for the mouse studies. These include THM-only, THM-wtSMC1, THM-mtSMC1, THM-wtNIP, and THM-scNIP. The CNGRC sequence was linked to the N-terminus of the peptides. Biotin was

labeled at the N-terminus of the fusion peptides in order for *in vivo* detection. Since it was possible that the THM sequence may have an inhibitory effect on NBS1-ATM interaction, we first tested the potential effect in PC-3 cells treated with the peptides/ irradiation (0 or 6Gy). 2 hours after IR, cells were harvested and co-immunoprecipitation experiments were performed using an anti-NBS1 antibody. The immunoprecipitates were then immunoblotted with anti-ATM or anti-NBS1 antibodies. Our data demonstrate that the THM sequence does not interfere with the NBS1-ATM binding (Figure 1). Therefore these peptides are



suitable to further studies.

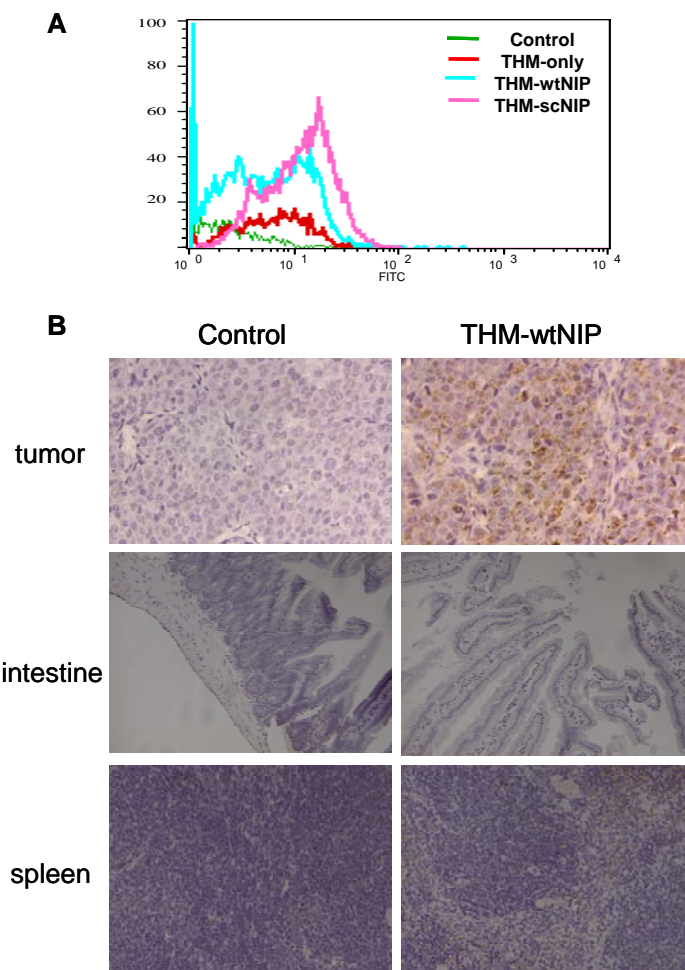
#### 2. Establishment of PC-3 xenografts.

The *in vivo* studies were performed in the Department of Cancer Therapeutics at the Drug Development Division of Southern Research Institute. Specific pathogen-free, 4-6 week old male nu/nu (nude) mice were obtained from Harlan, Sprague, Dawley and housed in sterilized filter-topped cages kept in laminar flow isolators. PC-3 tumor cells ( $2 \times 10^6$  per mouse in PBS) were

injected s.c. into the flanks of the mice. In all experiments, tumors were allowed to establish and grow before any treatment is initiated.

### 3. *In vivo* distribution of the peptides

When tumors reached approximately 100 mm<sup>3</sup>, animals were randomized and treated with the THM-only, THM-wtNIP, THM-scNIP, THM-wtSMC1, or THM-mtSMC1 peptides at doses ranging from 0.5-2 mg/kg by one of two routes: intraperitoneal (*ip*) or intra-tumoral injection (*it*). 0, 6, 12, or 24 hours after injection, the mice were euthanized, and the tumor tissue and normal tissues surrounding the tumor tissue were obtained. The samples will be assessed by a flow-activated cell sorting (FACS) analysis when stained with an FITC-conjugated streptavidin antibody. These experiments provided information on how fast the peptide could reach the tumor tissue, how long they would remain in the tumors, and whether or not the peptides would also accumulate in normal



**Figure 2. The THM-NIP fusion peptides home to the tumor tissue after *ip* injection.** Nude mice bearing PC-3 xenograft were injected (via *ip*) with 200μg of the THM-fusion peptides. 2 hours after injection, the tumor tissue, intestine and spleen were harvested and processed for **(A)** flow cytometry analysis using FITC-conjugated streptavidin or **(B)** immunohistochemistry using the Vectastain ABC system with the DAB peroxidase substrate to visualize peptide localization *in vivo*.

tissues. Localization of the peptides within tumor tissues was analyzed by dissection of the tumor tissues up to 24 hours after peptide injection. The tumor tissue specimen was stained with FITC-conjugated streptavidin, and immunofluorescence microscopy performed. We found that the tumor tissues injected with either route of the THM peptides showed at least of 80% of positive staining with an anti-streptavidin antibody, suggesting that the peptides can accumulate in tumor tissues (Figure 2A). Immuno-histochemistry experiments to investigate the localization of the THM peptides in tumor and normal tissues reveal that the THM-fusion peptides can specifically accumulated in tumor, but not in normal tissues (spleen and intestine) (Figure 2B).

#### **4. Xenograft radiation.**

We also performed preliminary experiments in order to obtain a radiation dose range for the xenograft studies. Mice with PC-3 xenografts reached 100 mm<sup>3</sup> were randomized and injected with peptides via *i.t.* Following a short interval to allow peptides to target tumors, mice were irradiated with a Precision X-RAD 320 Irradiation System. The dose rate for the irradiator at a distance of 50cm was 2.8Gy/min, while at a distance of 25 cm is 5.6 Gy/min. During the radiation procedure, mice were briefly (less than 5 minutes) restrained in a Plas Labs (Lansing, MI) clear plastic mouse-restraining device (tube) to allow the tumors to be targeted by radiation. Initial experiments have shown that 15Gy of local radiation was lethal to the majority of the mice, due to radiation damage to intestines. To over come this, we will 1) reduce the radiation dose to 10Gy, 2) shield the intestine area during radiation; and 3) reduce the area to be irradiated.

### **KEY RESEARCH ACCOMPLISHMENTS**

1. We have generated several THM-fusion peptides for *in vivo* studies;
2. The THM peptides have demonstrated tumor specific localization in PC-3 xenografts (Task 3a).

### **REPORTABLE OUTCOMES**

#### **A. Publications and meeting presentations:**

1. Xi Tang, Zhou-guang Hui, Xiao-li Cui, Renu Garg, Michael B. Kastan, and Bo Xu. A novel ATM-dependent pathway regulates Protein Phosphatase 1 and Histone H3 phosphorylation in response to DNA damage. *Molecular Cellular Biology*. April 4, 2008, 2559-2566

#### **B. Awards/honors:**

Xi Tang, postdoctoral fellow, AACR-WICR Brigid G. Leventhal Scholar Award for the 2008AACR annual meeting

#### **C. Invited seminars/lectures:**

1. "DNA damage response mechanisms, from fundamental biology to cancer drug discovery", Georgia State University, Atlanta, GA, December 7, 2007,
2. "Recent Advances in Cancer Drug Discovery", Samford University School of Pharmacy, March 12, 2008
3. "Modern Molecular Radiobiology: Molecular targeted Radiosensitization", Department of Radiation Oncology, The Methodist Hospital, Houston, TX. March 19, 2008

4. "DNA damage response mechanisms, from fundamental biology to cancer drug discovery", The Methodist Hospital Research Institute, Houston, TX. March 20, 2008
5. "Roles of ATM in DNA damage response", The 2nd International Conference on Frontiers in Biomedical and Environmental Health Sciences: DNA Repair and Cancer Biology. April 16-20, 2008, Hangzhou, China
6. "ATM and Breast Cancer", Tianjin Medical University Cancer Hospital, April 22, Tianjin, China
7. "DNA damage response and human diseases", Institute of Biophysics, Chinese Academy of Sciences, Beijing, China, April 23, 2008
8. "Damage response mechanisms and genetic instability", Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China, April 24, 2008
9. "Molecular Radiation Biology", Tianjin Medical University General Hospital, Tianjin, China, April 25, 2008
10. "Targeting DNA damage responses for cancer drug discovery", Nantong Biomix, Nantong, China, May 14, 2008
11. "Molecular Cancer Biology and Molecular Targeted Cancer Therapeutics", Nantong University Medical School, Nantong, China, May 15, 2008
12. "Targeting DNA damage pathways by small inhibitory peptides", 1st Annual World Congress of ibio2008, Hangzhou, China, May 19, 2008 (also served as session co-chair)
13. "DNA damage response mechanisms: implications for cancer drug development" UAB Comprehensive Cancer Center Experimental Therapeutics Seminar Series, November 22, 2008

## CONCLUSIONS

During the last performance period, we have generated several small inhibitory peptides that can interfere with DNA damage-induced signaling pathways mediated by ATM for *in vivo* studies. We have shown the *in vivo* tumor targeting ability of the THM-fusion peptides. We have also observed significant radiation damage to the intestine area after radiation. Experiments are ongoing to reduce the intestinal damage and evaluate the radiosensitization effects of the fusion peptides. We have been granted a six-month no-cost extension to complete the project.

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